Gene Expression Profile of the Human Trabecular Meshwork: NEIBank Sequence Tag Analysis

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Purpose. To characterize the gene expression pattern in the human trabecular meshwork (TM) and identify candidate genes for glaucoma by expressed sequence tag (EST) analysis as part of the NEIBank project.

Methods. RNA was extracted from dissected human TM and used to construct unamplified, un-normalized cDNA libraries in the pSPORT1 vector. More than 4000 clones were sequenced from the 5′ end. Clones were clustered and identified using GRIST software. In addition, the expression patterns of genes encoding olfactomedin-domain proteins were analyzed by RT-PCR.

Results. After non-mRNA contaminants were removed, 3459 independent TM-expressed clones were obtained. These were grouped in 1888 clusters, potentially representing individual expressed genes. Transcripts for the myocilin gene, a locus for inherited glaucoma, formed the third most abundant cluster in the TM collection, and several other genes implicated in glaucoma (*PITX2*, *CYP1B1*, and optineurin) were also represented. One abundant TM transcript was from the gene for the angiopoietin-like factor CTD6, which is located at on the long arm of chromosome 1, area 36.2-36.1 in the region of the glaucoma locus GLC3B, whereas other transcripts were from genes close to known glaucoma loci. The TM collection contains cDNAs for genes that are preferentially expressed in the lymphatic endothelium (matrix Gla protein, apolipoprotein D precursor, and selenoprotein P precursor). In addition to EST profiling, RT- PCR was used to detect transcripts of the olfactomedin-domain proteins latrotoxin receptor Lec3 and optimedin in the TM.

Conclusions. The TM libraries are a good source of molecular markers for TM and candidate genes for glaucoma. The abundance of myocilin cDNAs corresponds to the critical role of this gene in glaucoma and contrasts with libraries derived from cultured tissue. The expression profile raises the possibility that cells of the TM and Schlemm's canal may be more similar to lymphatic, rather than blood vascular endothelium. (*Invest Ophthalmol Vis Sci.* 2003;44:2588–2596) DOI:10.1167/iovs.02-1099

Glaucoma is a group of neurodegenerative disorders characterized by the death of retinal ganglion cells and by a specific deformation of the optic nerve head, known as glaucomatous cupping. Primary open-angle glaucoma, the most common form, is often associated with elevated intraocular pressure. In many cases of glaucoma elevated intraocular pressure develops as a result of abnormally high resistance to the outflow of aqueous humor.

The aqueous humor outflow system is located at the junction of the cornea and iris and consists of the trabecular meshwork (TM) and Schlemm's canal (SC), leading to the episcleral venous system. The TM and SC are complex structures composed of morphologically and functionally distinct cell types. In particular, the corneoscleral part of the TM consists of beams of connective tissues covered on both surfaces by endothelial-like cells.[1] The juxtacanalicular meshwork, which is located just below the SC, is a nontrabecular connective tissue containing three to five layers of star-shaped cells. Together with the inner wall of Schlemm's canal, this meshwork is thought to compose the region of main resistance to aqueous outflow.[2,3] This complexity is reflected in the differing embryonic origins of TM and SC cell types. Cells of the corneoscleral TM are derived from the neural crest, whereas cells of the juxtacanalicular meshwork may be derived from the perivascular cells of Rouget.[4] It is thought that the endothelial cells of the SC have a vascular origin.[5–7]

Mutation or altered expression of genes expressed in the TM could interfere with normal function of the tissue, thereby leading to glaucoma. Indeed, three genes associated with different forms of open-angle glaucoma, *CYP1B1*, myocilin (*MYOC*), and optineurin, are all expressed in human TM.[8–13] However, although expression of *MYOC* is higher in human TM and sclera than in other tissues,[9] none of these genes is tissue specific.

A catalog of the transcriptional repertoire of the TM would be of great value to increase our understanding of the function of the tissue and to help in the selection of candidate genes for inherited glaucoma. One powerful technique for investigating the expression profile of tissues or cell types is expressed sequence tag (EST) analysis.[14,15] The NEIBank project of the National Eye Institute has been undertaken to produce a molecular encyclopedia of the eye. As part of this effort, EST analyses of several human eye tissues have already been performed.[16–21] Until now, there has been little information available on the gene expression profile of native human TM. Gonzalez et al.[22] have described 833 clones from a PCR-amplified cDNA library constructed from the TM of perfused human eye. Among the first 20 most highly expressed genes, they noted genes encoding glycolytic enzymes (glyceraldehyde-3-phosphate-dehydrogenase [GAPDH], lactate dehydrogenase A [LDHA], and triosephosphate isomerase [TPI]), matrix GLA, chitinase 3-like 1, apolipoprotein D, small inducible cytokine (*SCYA20*), regulator of G-protein signal, stromelysin 1, and two uncharacterized genes *KIAA0258* and *DKFZp586O0118*. This

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Supported in part by the Fund for Research and Health of Quebec (FRSQ) Health Vision Research Network. VR was a FRSQ National Scientist.

Submitted for publication October 28, 2002; revised December 11, 2002; accepted January 22, 2003.

Disclosure: **S.I. Tomarev**, None; **G. Wistow**, None; **V. Raymond**, None; **S. Dubois**, None; **I. Malyukova**, None

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analysis surprisingly identified no clones for *MYOC* or for some other glaucoma-related genes, which suggests that culturing of tissue and amplification of mRNA may have produced a profile that does not closely match that of the native TM.

Herein, we present characterization of 3429 cDNA clones from unamplified cDNA libraries derived from freshly isolated human TM. Several genes implicated in glaucoma, including *MYOC*, optineurin, *PITX2*, and *CYP1B1*, are present in this library. The collection also contains cDNA for several potential candidate genes located close to known glaucoma loci in the human genome.

Materials and Methods

Tissue and RNA Preparation

Trabecular meshwork tissues were dissected from 28 human donors with no observed eye disease, less than 24 hours after death. The ages of the donors ranged from 54 to 87 years (mean, 71.4; median, 72). The collection of human eyes was approved by the Ethics Board of the Hospital Center of Laval University (CHUL). The procedure for obtaining the tissues was within the tenets of the Declaration of Helsinki.

The removal of TM tissues from the anterior angle is a delicate procedure. Rigorous measures were thus followed to minimize contamination of the dissected TM by surrounding tissues. In particular, eyes were not included in our study when there were difficulties in dissecting the TM from Schlemm's canal, iris, or cornea. Even though such procedures were used, a very small amount of cDNA clones derived from nearby cells may be present in our TM library.

The tissues were mixed in denaturing buffer (4 M guanidinium thiocyanate, 25 mM sodium citrate [pH 7] and 0.5% *N*-lauroylsarcosine) before phenol-chloroform extraction. The RNA was then precipitated using isopropanol, washed in 70% diethyl pyrocarbonate (DEPC)–treated ethanol, and resuspended in DEPC-treated water. RNA from each dissected tissue was analyzed separately by agarose gel electrophoresis to judge quality. Samples of good quality were combined, and 40 μg of total RNA was used for cDNA synthesis. Poly(A)$^+$ RNA was prepared using an oligo(dT) cellulose affinity column.

cDNA Library Construction

The cDNA, directionally cloned in the pSPORT1 vector (Life Technologies, Rockville, MD), was constructed at Bioserve Biotechnology (Laurel, MD). General details of library construction for NEIBank cDNA libraries are described elsewhere.[18–21] In this case, as an additional measure to remove small contaminant fragments, the cDNA was run over a resin column (Sephacryl S-500 HR; Gibco BRL, Grand Island, NY) designed to fractionate cDNA more than 500 bp. The columns were run in TEN buffer, containing 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, and 25 mM NaCl. Sublibraries (designated *bo*, *bp*, and *bq*) were made from the first three 35-μL fractions, containing cDNA.

cDNA Sequencing and Bioinformatics

Methods for sequencing and bioinformatics analysis are described in detail elsewhere.[19,21,23,24] Briefly, randomly picked clones were sequenced at the NIH Intramural Sequencing Center (NISC). Clones were sequenced from the 5′ end. A specially developed software tool, GRIST (Grouping and Identification of Sequence Tags) was used to analyze the data and assemble the results in Web page format.[25] Clusters of sequences were also examined (SeqMan II; DNAstar, Madison, WI) to determine alternative transcripts. Sequences were searched through genome resources at the National Institutes of Health (http://www.ncbi.nlm.nih.gov/) and the University of California at Santa Cruz (http://genome.ucsc.edu/; Human Genome Browser provided in the public domain by UCSC Genome Bioinformatics).

PCR Methods

Total RNA (1 μg) was used for cDNA synthesis with commercial reverse transcriptase (SuperScript; Gibco BRL) and oligo(dT)-primer. The amount of synthesized cDNA was evaluated by PCR using primers specific for cyclophilin (5′-TCCTGCTTTCACAGAATTATTCC-3′ and 5′-ATTCGAGTTGTCCACAGTCAGC-3′). PCR reactions were performed in a thermal cycler (PTC-200; MJ Research, Watertown, MA) with *Taq* polymerase (Ampli*Taq*; Applied Biosystems, Foster City, CA). Each PCR reaction was repeated at least twice. The thermal cycling parameters were as follows: 1 minute 30 seconds at 94° followed by 30 cycles of 25 seconds at 94°, 1 minute 30 seconds at 58°, and 1 minute at 72° and a final incubation for 5 minutes at 72°. PCR reaction products were analyzed by agarose gel-electrophoresis. After adjustment of cDNA concentration, relative abundance of mRNAs was estimated for myocilin (primers 5′-CTTATGACACAGGCACAGGTAT-3′ and 5′-GTGACCATGTTCATCCTTCTGG-3′), optimedin (5′-AGGCCTATCATGTCCTTGTCAT-3′ and 5′-CAGCACCGCATCAGAGAATTG-3′), olfactomedin-1 (5′-CCATTGCAGTGCCGTTTCTTG-3′ and 5′-ACTACGGCATTGCATTTACAACAA-3′), latrotoxin receptor Lec3 (5′-CCTCACTATATATCTTTATGCAGT-3′ and 5′-GACCTTCCAATGCTTACGAGG-3′), and olfactomedin-2 (5′-CCCTGTTTCACGTCATCAGCA-3′ and 5′-AACTGGAGAACCAGAGCCATAA-3′).

Results

Library Statistics

Column fractions of TM cDNA were cloned separately as libraries designated *bo*, *bp*, and *bq*. As in other NEIBank libraries,[19–21] all clones were numbered according to their library designation and their position in 96-well plates (e.g., hp07b10). For the *bo*, *bp*, and *bq* libraries, there were 1.3×10^6, 5.6×10^6, and 1.6×10^7 primary cDNA recombinants, respectively, with an average insert size of 500 to 700 bp. For each sublibrary, approximately 1200 clones were sequenced initially. All three had a very similar distribution of clones. An additional 1200 clones were then sequenced from the *bo* library, which seemed to have slightly lower levels of non-mRNA clones and a slightly larger average insert size. All the data were combined for subsequent analysis. In the combined data set, 3.7% of clones contained no inserts, and 16.3% contained a mitochondrial genome sequence. A total of 4518 quality 5′ reads from two libraries gave 3459 clones after removal of contaminants and very short sequences and masking of repetitive sequences. Analysis of these clones through GRIST[25] resulted in 1888 clusters potentially representing individual genes expressed in human TM. Of these clusters, 24% (n = 454) contained at least two clones, representing mRNAs that may be highly or moderately abundant in the human TM. The remaining sequences (n = 1434) appeared only once. Information about all sequenced clones was organized at the NEIBank Web site (http://neibank.nei.nih.gov/).

Gene Expression Profile of Human TM and Glaucoma Candidate Genes

Many of the most abundant cDNAs in the TM collection such as, vimentin, elongation factor 1α, translationally controlled tumor protein, and various ribosomal proteins, are also abundant in other cDNA libraries (Table 1). However among the most abundant TM cDNAs, there are also clones for two genes associated with glaucoma. *MYOC* is represented by 28 clones, corresponding to approximately 1% of the total sequenced. Until now, *MYOC* has been found at high abundance only in a subtracted human ciliary body cDNA library,[26] which suggests a significant tissue preference for this gene in TM.[27] Another gene, *PITX2*, which is the locus of Rieger syndrome, including malformation of the anterior chamber of the eye and glaucoma,[28] was also highly expressed in the human TM, being represented by six clones (Table 1). Among other abundant clones, those for the extracellular matrix Gla protein are also present at a frequency of approximately 1%. Transcripts for the

TABLE 1. The 50 Most Abundant cDNA Clones in the Human TM cDNA Libraries

Rank	Gene Name	GenBank	UniGene	Chromosome	*n*
1	Vimentin	M25246	29775	10p13	29
2	Elongation factor 1α	AK026650	181165	6q14	29
3	Myocilin	AF001620	78454	1q23	28
4	Tumor protein TPT1	NM_003295	279860	13q14.2	27
5	Matrix Gla	NM_000900	279009	12p12.3	22
6	Tropomyosin-α	M19715	77899	15q22.1	18
7	Ribosomal protein L41	Z12962	324406	12q13.3	15
8	Apolipoprotein D	J02611	75736	3q29	15
9	Purkinje cell protein 4	U52969	80296	21q22.2	14
10	Transmembrane E3-16	AF092128	239625	13q14.3	14
11	Nascent-polypeptide-associated	AF054187	32916	12q13.3	14
12	ATPase, Ca^{++}	NM_001681	1526	12q24.11	14
13	Ribosomal protein L9	AC006088	157850	15q25.2	14
14	Regulator of G-protein signaling	L13463	78944	1q31	13
15	Angiopoietin-like factor	XM_042319	146559	1p36	13
16	Ribosomal protein L31	NM_000993	184014	2q11.2	12
17	Ubiquinol-binding protein	M22348	131255	8q22	12
18	Ribosomal protein S27a	NM_002954	3297	2p16	12
19	Actin α-2	NM_001613	195851	10q23.3	11
20	Ribosomal protein S3A	NM_001006	77039	4q31.2	11
21	Decorin	L01131	76152	12q23	10
22	Ribosomal protein L6	NM_000970	349961	12q24.13	10
23	SPARC-like 1	NM_004684	75445	4q22.1	10
24	Thymosin β-4	XM_072582	75968	Xq21.3	10
25	Ribosomal protein S25	NM_001028	113029	11q23.3	10
26	Insulin-like growth factor binding protein 7	NM_001553	119206	4q12	10
27	Ribosomal protein L5	AF113210	180946	1p22	9
28	Ribosomal protein L37a	L22154	296290	2q35	9
29	Prothymosin α	XM_038341	250655	2q35	9
30	Ribosomal protein S24	NM_033022	180450	10q22	9
31	CREBBP/EP300 inhibitory protein 1	AF092135	75847	15q21.1	9
32	NADH dehydrogenase 1 α subcomplex, 5	NM_005000	83916	7q32	9
33	Ribosomal protein S20	NM_001023	8102	8q12	9
34	G protein, α stimulating activity polypeptide 1	NM_000516	273385	20q13.3	9
35	Ribosomal protein L7	NM_000971	153	8q13.3	9
36	v-Fos	NM_005252	25647	14q24.3	8
37	Ribosomal protein L23a	NM_000984	419463	17q11.2	8
38	Lysosomal-asociated protein transmembrane 4 α	NM_014713	111894	2p24.3	8
39	Ribosomal protein L27	L19527	111611	17p13.2	7
40	Cytochrome *c* oxidase VIIc	XM_003730	3462	5q14	7
41	Secreted calcium-binding protein 2	XM_051452	22209	6q27	7
42	Ribosomal protein L23	NM_000978	234518	17q21.1	7
43	Connective tissue growth factor	U14750	75511	6q23.1	7
44	Sterile-α motif and leucine zipper containing kinase AZK	NM_016653	115175	2q24.2	7
45	Phosphatase type 2B	AF043329	173717	1p32.2	7
46	PITX2	XM_045596	92282	4q25	6
47	Ribosomal protein L12	NM_000976	182979	9q34	6
48	Ribosomal protein S8	NM_001012	151604	1p34.1	6
49	Ribosomal protein L37	D23661	337445	5p13	6
50	Ribosomal protein S6	NM_001010	241507	9p21	6

This list is an edited detail from the output of the clustering procedure GRIST. Representative GenBank entries are shown, along with the current UniGene cluster, chromosomal location, and number of ESTs identified in the TM library.

same gene were highly abundant in cDNA libraries obtained from perfused human eye TM and from human corneal endothelial cells.[16,22]

Several TM cDNAs encode extracellular matrix (ECM) and cytoskeleton proteins. ECM may play an important role in maintaining normal aqueous outflow, and alterations to the ECM may lead to elevation of intraocular pressure and glaucoma.[1,29–32] Indeed, recent results indicate that myocilin, a secreted protein,[33–35] can associate with components of the ECM through interaction with fibronectin and fibrilin-1.[36,37] Table 2 lists the transcripts essential for the ECM assembly and function identified so far in the human TM collection. The previously identified components of the ECM in the normal human juxtacanalicular TM include fibronectin, laminin, elastin, decorin, and collagen I, IV, VI.[37,38] cDNAs for most of these proteins are present among the sequenced clones, with decorin being one of the most abundant clones in the TM library (Tables 1, 2).

The cytoskeleton of TM cells is also involved in the regulation of aqueous humor outflow, and drugs affecting the cytoskeleton network may reduce outflow resistance.[39] Table 3 lists the transcripts encoding cytoskeleton proteins identified so far in the human TM libraries. Vimentin clones constitute the most abundant cluster in the human TM collection and a significant fraction of other clones encode proteins involved in actin microfilament network organization and maintenance.

Overall, the relative abundance of cDNA clones in our collection from native TM and those obtained after PCR amplification of RNA isolated from TM of the perfused human eye were quite different. Only 4 of the 20 most abundant cDNAs in

TABLE 2. Extracellular Matrix Transcripts

Gene Name	GenBank	Chromosome	*n*
Myocilin	AF001620	1q23-q24	27
Matrix Gla protein	NM_000900	12p13.1-p12.3	22
Decorin	NM_001920	12q13.2	10
SPARC-like 1	NM_004684	4q22.1	10
Connective tissue growth factor	U14750	6q23.1	7
Osteopontin	AF052124	4q21-q25	6
Proteoglycan 1	NM_002727	10q22.1	4
TIMP2	AL110197	17q26.3	3
Osteonectin (SPARC)	XM_032759	5q31.3-q32	2
Chitinase 3-like 1	XM_015434	1q32.1	2
Semaphorin 3E	NM_012431	7q21.11	2
Sarcoglycan, epsilon	AF031920	7q21-q22	2
Collagen, type IV, alpha 3	NM_000091	2q36-q37	2
Laminin, beta 1	XM_027214	7q22	2
Lumican	NM_002345	12q21.3-q22	2
Matrix metalloproteinase 3 (stromelysin 1)	NM_002422	11q22.3	1
Matrix metalloproteinase 2, gelatinase A	J03210	16q13-q21	1
Collagenase type IV	J03210	16q13-q21	1
Collagen type IV alpha 5 chain	M31115	Xq22	1
Collagen, type III, alpha 1	NM_000090	2q31	1
Collagen alpha-2 type I	J03464	7q22.1	1
Collagen COLAIL precursor	AF330693	6p12.2	1
Fibronectin 1	AF130095	2q34	1
EGF-containing fibulin-like extracellular matrix protein 1	XM_002258	2p16	1

the library from the cultured eye (translation elongation factor, GLA, apolipoprotein D, and ribosomal protein L6) were found among the most abundant 50 cDNAs in the human TM library.

Glaucoma Genes

At least eight loci have been implicated in different forms of glaucoma, and so far three genes have been identified.[40] All three of these genes, *MYOC*, *CYP1B1*, and *OPTN*, are represented in the TM collection (Table 4). Other genes contribute to glaucoma as part of wider syndromes. As mentioned earlier, *PITX2*, the locus of Rieger syndrome is also represented by six cDNAs. Similarly, mutations in the wolframin gene encoding a transmembrane protein may lead to Wolfram syndrome, which in some cases is associated with juvenile glaucoma,[41] and a single cDNA for wolframin is in the TM collection.

The TM collection also includes many cDNAs from genes (many of unknown function) that are located close to glaucoma loci and as such may be considered candidate genes (Table 4). Considering the example of *MYOC*, which is not TM-specific but is highly expressed in the tissue, *CTD6* is an interesting potential candidate. The *CTD6* gene is located close to the locus for GLC3B and is one of the 15 most abundant transcripts in the TM collection, at a frequency of approximately 0.5%. Another relatively abundant cluster of cDNA

TABLE 3. Cytoskeleton-Related Transcripts

Gene Name	GenBank	Chromosome	*n*
Vimentin	M25246	10p13	29
Skeletal muscle alpha-tropomyosin	M19715	15q22.1	18
Actin, alpha 2, smooth muscle	NM_001613	10q23.3	11
Thymosin, beta 4	XM_099025	Xq21.3	10
ARGBP2-like	AK056758	4q35.2	8
Palladin	AF077041	4q32.3	6
Destrin	NM_006870	20p11.23	4
Myosin, light polypeptide 6	NM_021019	12q13.13	3
Desmuslin	XM_031031	13	3
ArgBP2 (splice variant)	NM_021069	4q35.1	2
K12 keratin	D78367	17q12	2
Caldesmon	AJ223812	7q33	2
Calponin 3, acidic	XM_001324	1p22-p21	2
Arp2/3 protein complex subunit p34-Arc	AF006085	13q12-q13	2
Gelsolin	NM_000177	9q33	2
Actin, beta	XM_098710	7p22.1	2
Cortactin-binding protein 2	AF377960	7q31.31	2
Arp2/3 protein complex subunit p21-Arc	AF006086	12q24	1
Actin binding protein MAYVEN	AF059569	4q21.2	1
Alpha-actinin-2-associated LIM protein	AF039018	4q35	1
Microtubule-actin crosslinking factor 1	NM_012090	1p32-p31	1
Cofilin 2	AF134802	14q13.2	1
Suppressor of profilin/p41 of actin-related complex 2/3	BC006445	7q22.1	1
CAPZA1	XM_052116	1p13.1	1
Ems1 (cortactin)	XM_006181	11q13	1

Table 4. Known and Candidate Genes for Glaucoma

Gene Name	GenBank	Glaucoma Locus	Chromosome	*n*
Known genes				
MYOC	NM_000261	GLC1A	1q24.3	27
CYP1B1	NM_000104	GLC3A	2p22.2	2
OPTN	NM_21980	GLC1E	10p14	1
Candidate genes				
		GLC1B	2cen-q13	
RANBP2	NM_006267 1			
ECRG4	XM_030022			1
MGC3062	AF267853			1
LOC51239	AF151034			1
LOC150587	XM_097917			1
		GLC1C	3q21-24	
LOC152017	XM_098153			6
MLCK	AB037663			2
ZNF9	M28372			4
HSPC056	NM_014154			2
LOC152215	XM_087407			2
DKFZp434A045	AL137629			2
FAIM	BC012478			2
AMOTL2	AF175966			1
PLSCR4	XM_002843			1
DBR1	AF180919			1
ATP1B3	NM_001679			1
FLJ22897	AK026550			1
LOC152154	XM_098168			1
SNX4	XM_027161			1
TM4SF1	NM_014220			1
LOC131341	XM_067332			1
H41	AF103803			1
MBD4	AF072250			1
RNF7	AF164679			1
TRAD	AB011422			1
RYK	NM_002958			1
IMAGE:4616798	BC020883			1
PIK3R4	XM_030812			1
MRPL3	NM_007208			1
FLJ23251	AL136757			1
ITGB5	XM_003029			1
FLJ37613	AK094932			1
		GLC1D	8q23	
EBAG9	NM_004215			2
FLJ20366	AB040905			1
COX6C	NM_004374			1
DC6	AF173296			1
		GLC1F	7q35-36	
RHEB2	NM_005614			2
INSIG1	NM_005542			1
		GLC3B	1p36.2-p36.1	
CTD6	NM_021146			13
RERE	XM_045241			3
RPL11	NM_000975			3
PMSCL2	NM_002685			2
P29	AF273089			2
PINK1	AB05323			1
AL137274	XM_072050			1
FLJ10199	BC000593			1
LOC126913	XM_059093			1
EIF4C	L18960			1
EIF4G3	XM_027919			1
MIG6	XM_097469			1
HNPR	AF000364			1
FLJ10521	BC003073			1
MFN2	AF036536			1
		GLC3C	14q24.3	
c-FOS	NM_005252			8
NPC2	NM_006432			2
LTBP2	NM_000428			1
SFRS5	NM_006925			1
LOC57862	NM_021188			1
MLH3	XM_040415			1
FLJ22042	AK025695			1
S164	XM_027330			1
FLJ13553	L40391			1
IMAGE:5503603	BM466487			1

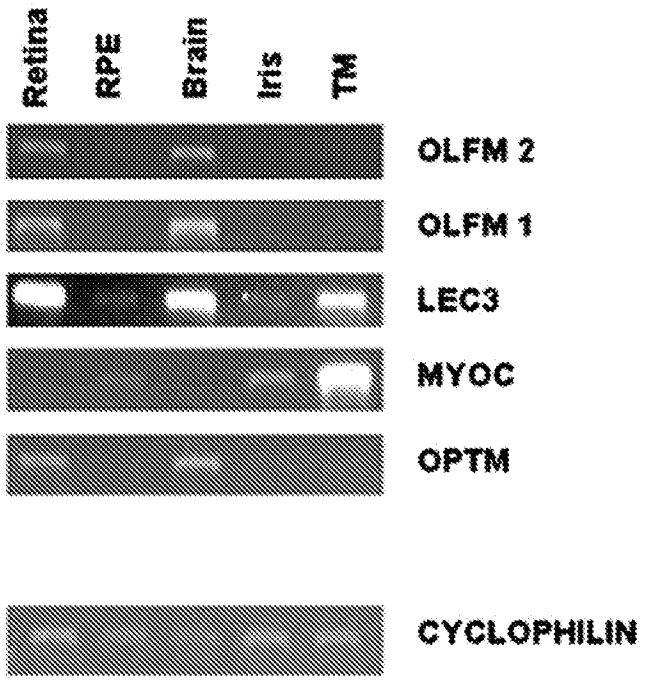

FIGURE 1. Expression of genes encoding the olfactomedin-containing proteins in different human eye tissues and brain determined by semiquantitative RT-PCR. cDNAs from the indicated tissues were used as templates.

clones is a group of six that correspond to a hypothetical gene with the National Center for Biotechnology Information (NCBI) designation *LOC152017*. Examination of the location of these transcripts in the human genome suggests that they represent a long 3′ untranslated region (UTR) of the myosin light chain kinase (*MLCK* or *MYLK*) gene, which is itself represented by two other ESTs in the TM collection, corresponding to the sequenced cDNA in GenBank (http://www.ncbi.nlm.nih.gov/Genbank; provided in the public domain by NCBI, Bethesda, MD). It seems likely therefore that the TM collection contains at least eight ESTs for this gene, which is located close to GLC1C. *ZNF9* is another gene close to the GLC1C locus and is represented four times in the TM collection.

Expression of Genes Encoding the Olfactomedin Domain in the Eye Tissues

To validate the high levels of expression of *MYOC* in TM implied by the abundance of the cDNA, RT-PCR was used. We have demonstrated that myocilin may interact with another olfactomedin-containing protein, optimedin, through the olfactomedin domain.[35] In the collection of cDNAs sequenced for this study, no clones for other known olfactomedin domain-encoding genes were present, and RT-PCR was therefore also used to examine the expression pattern of some olfactomedin-encoding genes in several eye tissues and brain (Fig. 1).

As expected, *MYOC* was highly expressed in the TM and was also detected in the iris and RPE. Optimedin and olfactomedin were expressed in brain and retina. Weak expression of optimedin was also detected in the TM. A latrotoxin receptor Lec3[42] was expressed in all tissues analyzed, with highest expression in brain, retina, and TM.

DISCUSSION

In the present study, we constructed cDNA from carefully dissected normal human TM and characterized the gene expression pattern by EST analysis. Although a cDNA library has been constructed from the TM of a perfused human eye, this is the first report of an unamplified cDNA library obtained from native human TM. In many cases, the gene expression profiles of native tissue and cultured cells derived from the tissue are different. For example, the relative abundance of cDNAs present in cDNA libraries obtained from corneal endothelial cells[16] or RPE[17] is quite different from that present in (respectively) corneal endothelial[43] and RPE[44,45] cell lines. This is also true of TM. For example, cDNAs for *MYOC* and *PITX2* were abundant in the EST collection from native TM but were not present at all among the clones sequenced from the library made from TM of perfused eyes. Transcripts for the glycolytic enzymes LDHA, GADPH, and TPI were among the 12 most abundant clones in the library from perfused eye.[22] cDNAs for these genes were found in the native TM library, but at lower relative levels.

Several cDNA libraries from different eye tissues having different embryonic origin and function have recently been characterized.[16,18–21,44] Some of these might be expected to share some similarities with the TM. For example, the filtering cells of the TM share a neural crest origin with corneal endothelial cells. However, although cDNAs corresponding to ribosomal proteins involved in protein synthesis are abundant in both human corneal endothelial cells[16] and TM, the complement of other abundant clones is quite different in the two tissues. Prostaglandin D_2 synthase is the most prevalent transcript in the corneal cDNA library, and only cDNAs for matrix Gla protein and translationally controlled tumor protein were present in the list of the abundant clones in both libraries.[16] It is noteworthy that mRNA sequences for another extracellular matrix protein, decorin, were abundant in the human TM library and human Fuchs' corneal endothelial SAGE library (see UniGene Cluster Hs. 76152; http://www.ncbi.nlm.nih.gov/UniGene; provided in the public domain byNCBI). The iris is another specialized tissue of the anterior segment of the eye in proximity to the TM. The iris contains several different cell types, plays a critical role in eye development and function, and may also be involved in several eye disorders including glaucoma.[46] Although there is overlap in the expression of many genes between the TM and the iris, the lists of the most abundant transcripts in the two tissues are different (see Table 1 in this article and Table 1 in Ref. 19). Differences in the most abundant transcripts between the iris, cornea, and TM serve as another indication that the TM samples used for cDNA library construction were not significantly contaminated by the surrounding tissues.

MYOC clones were identified in human iris and also in RPE/choroid cDNA libraries but not in the retina or lens cDNA libraries. This agrees with the results of RT-PCR described herein, in which *MYOC* was amplified from iris and RPE/choroid, but not from retina or lens (Fig. 1). The high abundance of *MYOC* transcripts in TM compared with other tissues in the eye and elsewhere correlates with the observation that glaucoma-causing mutations in this gene do not lead to disease elsewhere in the body. Myocilin contains an olfactomedin domain, through which it can interact with other olfactomedin domain proteins. The present collection of TM cDNAs does not contain any clones for any other identified proteins of this family. However, we checked for the expression of some other olfactomedin domain genes by RT-PCR. In the human eye, expression of *MYOC* corresponds most closely with that of the latrotoxin receptor, *Lec3*.[42] It is interesting that cDNAs for both myocilin and another member of the latrotoxin receptor family, lectomedin, have been detected in the human fetal cochlea (http://neibank.nei.nih.gov/). We are now testing possible interactions between the two proteins. In the rat eye, optimedin, another olfactomedin domain protein, is strongly

expressed in the tissues of the eye angle, retina, and brain. In human tissues, expression of optimedin in the tissues of the angle was significantly weaker than in the retina or brain. Indeed, our preliminary data indicate that there are significant variations in the level of expression of olfactomedin-containing genes between rat, mouse, and humans. This may have significance for animal experimental models of diseases involving these proteins.

The endothelial cells of Schlemm's canal and some cells in the juxtacanalicular TM may have a vascular origin.[4–7] There are two vascular systems in humans: the lymph vascular system and the blood vascular system. One of the main functions of the lymphatic system is to remove an excess of erythrocyte-free, protein-rich interstitial fluid that escapes from blood capillaries and return it to blood circulation. In many respects, this is reminiscent of the aqueous outflow system of the eye. The aqueous humor has similarities with lymph and the TM closely resembles a lymphatic sinusoidal network. Schlemm's canal has an endothelial lining and transports the aqueous humor toward the blood, thereby functioning like a lymphatic collector. A divergent homeobox gene *Prox1* is essential for development of the lymphatic system in mice[47] and is a marker of lymphatic endothelial cells in humans.[48,49] Antibodies against the Prox1 protein stain lymphatic but not blood vessels. Although *PROX1* cDNA was not present among the sequenced clones in the human TM library, expression of the *PROX1* gene has been detected in the human TM by RT-PCR (Malyukova I, Tomarev SI, unpublished data, 2002). Recent data demonstrate that overexpression of *PROX1* in blood vascular endothelial cells in vitro induces expression of lymphatic vascular endothelial cell-specific markers and suppresses the expression of approximately 40% of the blood vascular endothelial cell-specific genes.[50] Several genes that are preferentially expressed in the lymphatic versus blood vessel endothelium (matrix Gla protein, apolipoprotein D precursor, and selenoprotein P precursor)[50] were found abundantly in the human TM cDNA collection (Table 1). On the basis of these observations, we suggest that endothelial cells of the Schlemm's canal and at least a population of TM cells may be more similar to the lymphatic endothelial cells than to the blood vascular endothelium cells.

The TM cDNA collection gives a view of part of the transcriptional repertoire of the tissue. Among these expressed genes are likely to be the loci for inherited TM-related disease and, indeed, the collection contains cDNAs for three known genes associated with glaucoma. Table 4 lists a number of genes close to known glaucoma loci that are represented in our collection. One interesting candidate is *CDT6*, which is located at 1p36.22, within the glaucoma locus GLC3B, and is among the 15 most abundant cDNAs in the TM collection (Table 1). *CDT6* encodes a secreted angiopoietin-like factor that is also highly expressed in human corneal stroma.[51,52] Angiopoietins are the ligands for the vascular endothelial Tie2 receptors, and they are involved in vascular morphogenesis and maintenance. Several blinding diseases, including neovascular glaucoma, are related to an aberrant angiogenic response.[53] The angiopoietin/Tie signaling pathway is considered to be involved in cell migration, proliferation, and survival, and reorganization of the actin cytoskeleton.[54] However, CDT6 does not bind the Tie2 receptor, indicating that it does not function as a true member of the angiopoietin family.[55] It has been suggested recently that expression of *CDT6* may stimulate the deposition of specific extracellular matrix components and that CDT6 is a morphogen for human cornea.[52] Alterations of extracellular matrix have been implicated in the pathogenesis of primary open-angle glaucoma.[29,32,56] It is easy to imagine that mutations in the *CDT6* gene could have profound effects on the TM leading to glaucoma. In the TM library, the most abundant cDNAs for extracellular matrix proteins correspond to myocilin, GLA, decorin, SPARC-like 1, and osteopontin (Table 2).

Another candidate represented in among the TM cDNAs is the gene for the RERE protein located at 1p36.23. RERE contains an arginine-glutamic acid dipeptide repeat and is able to interact with the DRPLA protein, which contains a glutamine repeat and is involved in dentatorubral-pallidoluysian atrophy.[57] Although no connection between DRPLA and glaucoma is known, it has been shown that DRPLA is associated with corneal endothelial degradation.[58] Other abundant cDNAs from genes in the 1p36 region include myosin light chain kinase (*MYLK*) and *ZNF9*. *MYLK* has two products, the light chain kinase and a truncated version, telokin, and controls contractile activity in smooth muscle.[59] *ZNF9* encodes a zinc finger protein that binds to sterol regulatory elements and is the locus for myotonic dystrophy 2 (Online Mendelian Inheritance in Man [OMIM], 602668), a condition that includes cataract.[60,61]

Mutations in two mouse genes, *Gpnmb* and *Tyrp1* were recently implicated in iris pigment dispersion and iris stromal atrophy,[46,62] although no mutations were found in several human families with pigment dispersion syndrome.[62,63] cDNAs corresponding to *TYRP1* and *GPNMB* genes were present five and four times, respectively, among the sequenced TM clones and were among the most abundant 100 cDNAs in the TM library. These data may justify further screening of families with glaucoma for mutations in these genes.

It is well documented that there are circadian changes in IOP.[64] Although the molecular mechanisms of mammalian circadian rhythm has not been fully clarified, mammalian Per proteins are thought to be key players. cDNA for *PER1* was present once among the sequenced clones in the TM library. In addition, the collection includes three clones for KIAA0443, an uncharacterized gene product that is similar to the rat PER-interacting protein, PIPS. It has been suggested that Pips might be involved in the feedback loop or output mechanism of circadian rhythm through interaction with Per1.[65] The TM collection also contains one clone for the transcription factor DEC-2, which has recently been shown to play a role in regulating the mammalian circadian clock.[66]

In conclusion, the identification of genes expressed in the human TM will help to elucidate the molecular mechanisms involved in normal function of the TM as well in TM disease. The TM cDNA collection identifies several interesting candidate genes for inherited glaucoma. In addition, the expression profile of TM raises the possibility that the human eye outflow pathway has some similarities to the lymphatic system.

Acknowledgments

The authors thank Jeff Touchman and Gerry Bouffard of NISC; Don Smith, Patee Buchoff, and James Gao of NEI for support in sequencing and organizing data; and I. Rodriguez of NEI for RNA samples from human retina and RPE. The authors also acknowledge Ghislaine Chamberland, Francine Simard, and Ide Dubé of the CHUL Eye Bank for their generous contribution to this work.

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